

Monitoring Occupational Exposure to Ethylene Oxide by the Determination of Hemoglobin Adducts

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In a study on workers in a chemical plant where ethylene oxide (EtO) is manufactured and partly used for ethylene glycol production, exposure to EtO was monitored during annual periodic health assessments in January 1988, December 1988, and March 1990 by the determination of the level of 2-hydroxyethylvaline (HOEtVal) in hemoglobin. The HOEtVal levels in workers corresponded with the potential EtO exposures. The highest level was found in December 1988, in blood samples collected 1-2 months after a shut-down, maintenance, and start-up program. The range of adduct levels found in the three examinations indicated that average EtO exposures during the 4 months preceding blood sampling were below 0.5 ppm. It was demonstrated that the method allows for the accurate monitoring of low levels of EtO exposure and provides personalized time-integrated exposure data with great discriminative power. In addition, the method may serve to identify unexpected personal exposures, which may lead to targeted exposure control measures.

Introduction

In the last decade, chemical techniques have been developed for the quantitative determination of the amount of adduct formed by reaction of ethylene oxide (EtO) with specific amino acids of hemoglobin, e.g., the N^ε and N^α-atoms of histidine and the free amino group of N-terminal valine. These techniques have been applied to the biological monitoring of human exposure to EtO (1-4). In the present paper, the biological monitoring of EtO exposure by the determination of 2-hydroxyethyl adducts to the N-terminal valine in hemoglobin of workers engaged in the manufacture of EtO over 3 consecutive years is discussed.

Methods

Study Population

The study was carried out on workers of the EtO plant of Shell Nederland Chemie, Klundert, The Netherlands, where EtO is manufactured and partly used for ethylene glycol production. For hemoglobin adduct analyses, blood samples were collected during the voluntary periodic health assessments in January 1988,

December 1988, and March 1990. In January 1988, blood was collected from 39 workers, in December 1988 from 41 workers, and in March 1990 from 48 workers. During the study period there was a regular exchange of workers to other chemical plants of the site. From two workers who were transferred, for non-medical reasons, to other chemical plants without EtO exposure on April 1, 1989, several blood samples were collected between April and July 1989 to study the decline of the hemoglobin adduct.

From previous studies it is known that persons not occupationally exposed to EtO have background levels of adducts, which are related to cigarette smoking in particular (5). For concurrent comparison of adduct levels of EtO-exposed workers, blood samples from control groups were collected and analyzed during the same periods as the EtO-exposed groups. A questionnaire was used to collect data on smoking habits from each subject, both in the EtO-exposed and the control groups.

Description of Ethylene Oxide Exposures

It is believed that the level of EtO-hemoglobin adducts reflects integrated exposures over the life span of the red blood cell, i.e., about 120 days in humans. For the interpretation of measured adduct levels, it is therefore important to have information about potential exposures to EtO during the 4 months preceding blood sampling.

In the periods October 1987-January 1988 and December 1989-March 1990, the plant was operating under normal conditions. However, in the September/October 1988 there was a shut-down, maintenance, and start-up program, during which po-

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tential EtO exposures might have been higher than under normal operating conditions. Measurements during normal plant operations showed that 8-hr airborne EtO exposures were below 0.5 ppm. During the shut-down-start-up period, 8-hr measurements were not carried out.

Workers were trained to self-monitor their short-term EtO air exposures using 3M badges (model 3551 EtO monitors) during activities when peak exposures to EtO might occur, e.g., exchanging and cleaning filters, repair work on pumps. During these activities, air-supplied respirators were always available. Sampling times varied mostly between 15 and 60 min. In the fourth quarter of 1987, EtO levels ranged from 0.5 to 14 ppm ($n = 33$), in the same period of 1988 they ranged from 1 to 8 ppm ($n = 36$), and in the first quarter of 1990 they ranged from < 0.5 to 1.7 ppm. Airborne EtO levels were measured outside the respiratory equipment and therefore may not reflect individual inhalational EtO exposures.

Hemoglobin Adduct Analyses

In the present study, the amount of 2-hydroxyethylvaline (HOEtVal) in hemoglobin was determined using the radio-immunological technique (RIA) developed and validated by Wraith et al. (3). For comparison of results, the amount of HOEtVal was also determined using the modified Edman degradation procedure and GC-MS analyses (6) for all blood samples collected in January 1988 and for a selection of samples collected in December 1988 and March 1990. Only the GC-MS was applied to determine HOEtVal in hemoglobin of the two workers from whom multiple samples were taken between April and July 1989 after the workers were transferred to another plant.

Blood samples were collected in heparinized vacuum tubes, and red blood cells were centrifuged, washed with saline, and lysed with water. The hemolysate was dialyzed for 48 hr against distilled water and the globin isolated by precipitation with ethyl acetate from a solution of 2-propanol containing 0.5% HCl/hemolysate (6:1). Ten-milligram globin samples were used for the RIA and 50 mg for the modified Edman degradation procedure and GC-MS analyses. With the RIA, the limit of detection was 25 pmole/g hemoglobin and the within-run relative standard deviation (RSD) was 5% at the level of 200 pmole/g hemoglobin. To measure analytical variations over time, a selection of globin samples collected and analyzed in January 1988 were stored at -70°C and reanalyzed during the runs of December 1988 and March 1990. Similarly, globin samples collected in December 1988 were reanalyzed during the run of March 1990. GC-MS analyses of the pentafluorophenylthiohydantoin of HOEtVal (HOEtVal-PFPTH) were carried out with the instrument (VG TS-250 or Finnigan TSQ 4500) in the chemical ionization mode, using methane as reagent gas. The limit of detection was 1–10 pmole/g hemoglobin and the within-run RSD 5–10% at levels between 500 and 1000 pmole/g hemoglobin. Analyses of the HOEtVal-PFPTH in the multiple samples collected from two workers between April and July 1989 were carried out with a HP 5971 mass selective detector in the electron impact mode. The limit of detection was 20 pmole/g hemoglobin and the within-run RSD 10% at a level of 25 pmole/g hemoglobin and 1.1% at a level of 800 pmole/g hemoglobin.

Results and Discussion

For the three control groups examined in January 1988, December 1988, and March 1990, statistically significant correlations were found between the number of cigarettes smoked per day and HOEtVal levels. On the average, smoking 10 cigarettes per day was found to increase the HOEtVal level by 61, 60, and 73 pmole/g hemoglobin, respectively (RIA method).

Average HOEtVal levels in nonsmoking controls were 43, 76, and 188 pmole/g hemoglobin, respectively. This systematic increase of background levels in nonsmoking controls was due to analytical variations in the RIA over time, as shown by similar increases of HOEtVal levels in samples collected and analyzed in January 1988 and reanalyzed in December 1988 and March 1990. To calculate the increment of the amount of HOEtVal in hemoglobin due to occupational EtO exposure, from each worker's measured HOEtVal level, the average background level of HOEtVal found in persons in the concurrently analyzed control group, matched for the number of cigarettes smoked per day, was subtracted. The results of the increment from EtO exposure is shown in Figure 1. The highest increment was found in December 1988 (median: 238 pmole/g hemoglobin; range 15–1200), followed by January 1988 (median: 145 pmole/g hemoglobin; range 0–1269), and March 1990 (median 53 pmole/g hemoglobin; range 0–437). Overall, there was a good correlation between the results obtained using the RIA and the GC-MS methods. For HOEtVal levels analyzed in January 1988, the relationship is expressed by the equation $y = 0.96x - 29$ ($n = 39$) with a correlation coefficient of 0.971 ($y = \text{RIA}$ and $x = \text{GC-MS}$), and for analyses in December 1988 by $y = 0.92x - 29$ ($n = 25$), with a correlation coefficient 0.994.

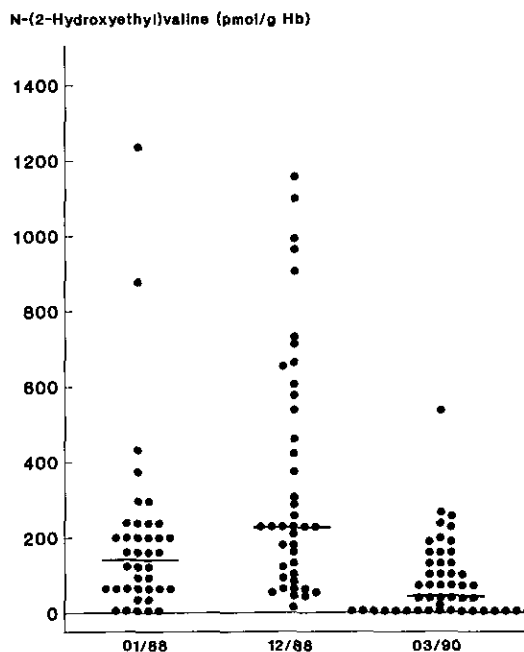


FIGURE 1. Levels of N-(2-hydroxyethyl)valine in hemoglobin of workers in the ethylene oxide manufacturing plant. Blood samples were collected during periodic health assessments in January 1988, December 1988, and March 1990. The horizontal bars represent median N-(2-hydroxyethyl)valine levels.

No equation was established for analyses in March 1990 because the number of samples analyzed both by RIA and GC-MS was too small. In a recent Swedish study, it is claimed that a time-weighted average exposure of 1 ppm, 40 hr/week during 4 months corresponds with 2400 pmole HOEtVal/g hemoglobin (7). The range of adduct levels in the present study indicates that the 4-month average 8-hr time-weighted EtO exposures ranged from 0 to 0.46 ppm (median: 0.10 ppm) in December 1988, 0–0.49 ppm (median 0.056 ppm) in January 1988, and 0–0.17 ppm (median 0.020 ppm) in March 1990. The median levels were clearly below the occupational exposure limit of 1 ppm (8-hr time-weighted average) set by the American Conference of Governmental Industrial Hygienists, 1990–1991 (9). The variations of HOEtVal levels during the years 1988–1990 can be explained by a combination of factors, such as plant operating conditions and differences in work practices of operators. Analytical variations were compensated by the use of control groups.

From the results of the short-term EtO air exposure measurements (by self-monitoring), it is clear that potential exposures to EtO could occur if personal protection equipment is not or inadequately worn. After the results of January 1988, campaigns were started to improve individual work practices, e.g., better use of available air-supplied breathing apparatus or activated carbon filters during short-term EtO exposures. Nevertheless, the increment of HOEtVal nearly doubled in December 1988 compared to January 1988. This was due to EtO exposures during a shut-down, maintenance, and start-up program 1–2 months before blood sampling, during which EtO exposures were increased compared to normal operating conditions. The effect of the campaigns to reduce personal EtO exposure during normal operating conditions became clear in March 1990, when much lower adduct levels were found. Analyses of the adduct data also revealed large variations between workers of the five shifts and within a shift. These variations could also be explained by differences in work practices. For example, the highest increment of adduct levels in both January and December 1988 was seen in workers of a shift who were mostly engaged in repairing EtO leakages. Other factors, such as individual variations in toxicokinetics of EtO, may also have contributed to the variations of HOEtVal levels between workers.

From work in experimental species, it is believed that formed HOEtVal adducts in hemoglobin are stable and that the rate of decline corresponds to the rate of replacement of red blood cells (about 120 days in humans). In the present study, we had the opportunity to analyze blood samples from two nonsmoking workers at several time intervals after transfer to another plant without EtO exposure. Figure 2 shows that the decline was linear in a semilogarithmic plot, from which biological half-lives of the HOEtVal adduct of 27 days (worker 1) and 35 days (worker 2) were calculated. Adduct levels were estimated by extrapolation 120 days after transfer to be 27 and 10 pmole/g hemoglobin, respectively, which are typical background adduct levels for nonsmokers (8). These results suggest that the adduct is being removed from the blood as the red blood cells degenerate.

The conclusions of this study are a) the determination of HOEtVal in hemoglobin is a sensitive method for monitoring time-integrated low levels of EtO exposure. In nonsmokers an increment of 50 pmole/g globin due to occupational EtO exposure can easily be determined, particularly when using GC-MS. It is

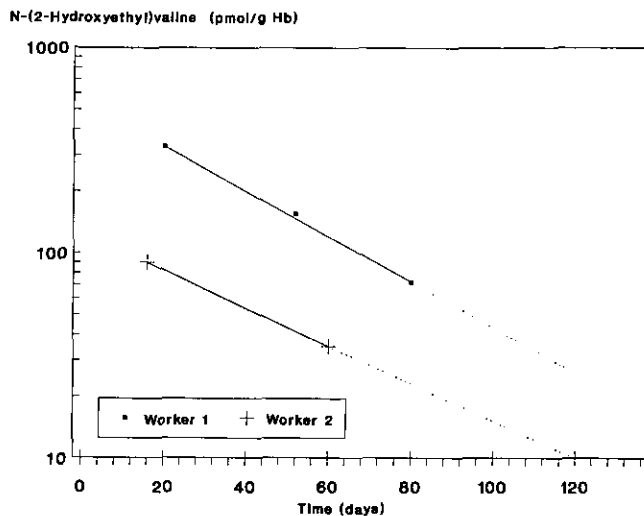


FIGURE 2. Levels of *N*-(2-hydroxyethyl)valine in hemoglobin of two non-smoking workers after they were transferred from the ethylene oxide manufacturing plant.

currently believed that such an increment corresponds with an average EtO exposure level of 0.02 ppm (40 hr/week). However, cigarette smoking and analytical variations as seen with the RIA in March 1990 samples are confounding factors, causing a decrease of the discriminatory power of adduct measurements at low EtO exposures. b) The lifetime of the adduct is similar to the life span of the red blood cell (120 days). c) The determination of HOEtVal in hemoglobin may serve to identify unexpected personal exposures, which may lead to targeted exposure control measures.

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